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Impact of diet on faecal output and caecotroph consumption in rabbits

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Structured Summary

Objectives: To assess the impact of four rabbit diets (hay only; extruded diet with hay; muesli with hay; muesli only) on faecal pellet size, faecal output and caecotrophy.

Methods: Thirty-two Dutch rabbits were studied over 17 months. Faecal pellet size and weight were measured in weeks 3, 9, 21 and 43 and faecal output in weeks 10, 22 and 45. Number of uneaten caecotrophs was recorded weekly.

Results: Faecal pellets were consistently smaller and lighter in rabbits fed muesli only, and the size of pellets produced by those fed muesli with hay decreased over the course of the study. Faecal output was greatest in rabbits with the highest hay intake. Uneaten caecotrophs were found in greatest frequency in rabbits fed muesli.

Clinical Significance: Muesli diets have a negative effect on faecal output and caecotroph ingestion and may therefore predispose to digestive conditions. Higher hay intake is associated with greater faecal output and less uneaten caecotrophs and may assist in preventing gastrointestinal stasis.

Keywords: Rabbit, Digestive, Gastrointestinal Stasis, Caecotrophs, Fly strike

Introduction

Whilst rabbits are popular pets (PFMA 2015; PDSA 2015), studies to determine their specific nutritional requirements have not been conducted. Published nutritional guidelines (NRC 1977; FEDIAF 2013) are based on research conducted on laboratory and commercially farmed rabbits. Pet rabbits were traditionally fed muesli-type diets however the number fed in this way swas reduced with more being fed hay, pelleted foods and vegetables (PDSA 2015). Muesli-type diets, especially if fed without hay, have been suggested to play a role in a range of disease processes including gastrointestinal stasis (Mullan and Main 2006; Meredith 2012; RSPCA 2013), and previous studies have found that they are also associated with obesity (Prebble et al. 2015a), dental disease (Meredith et al. 2015), and behavioural changes (Prebble et al. 2015b).

Dietary fibre particles are separated in the proximal colon of the rabbit according to their size (Jilge 1982), with small particle (<0.3mm) retained in the caecum for microbial fermentation (Gidenne 1993) and larger particles passed rapidly through the colon to be expelled in faecal pellets. Larger fibre particles are necessary to maintain normal caecal-colic motility (Cheeke 1994). Increasing dietary fibre levels increases the rate of passage of ingesta (Hoover and Heitmann 1972; Fraga et al. 1991; Gidenne 1992; Bellier and Gidenne 1996) conversely fine grinding of fibre results in prolonged retention times and reduced food intake (Laplace et al. 1977; Fraga et al. 1991; Gidenne 1992). The caecal contents are expelled once or twice in a 24-hour period as mucus coated caecotrophs that are consumed intact directly from the anus (Madsen 1939; Taylor 1939). Caecotrophy provides a source of B complex vitamins, vitamin K and microbial protein (Kulwich et al. 1953; Hirakawa 2001). The amount of caecotrophs consumed is influenced by diet; all are eaten when food is scarce, but when food is provided *ad libitum* protein and fibre content affect consumption. Increased levels of dietary fibre increase consumption and high protein levels reduce consumption. Increased dietary fibre content also reduces the protein and volatile fatty acid content of caecotrophs (Carabaño et al. 1988).

Gastrointestinal (GI) disorders are common in pet rabbits (Varga 2014); Harcourt-Brown 2014)) and are often considered to be related to diet rather than enteric pathogens. GI stasis is characterised by progressive hypomotility and eventual cessation of intestinal movement with clinical signs including anorexia, reduced/absent faecal production and accumulation of gas within the digestive tract (Harcourt-Brown 2002; Oglesbee and Jenkins 2012). Faecal pellets gradually reduce in size and frequency of production and may also be misshapen (Saunders and Rees Davies 2005). Pet rabbits also commonly present with uneaten caecotroph accumulation around the anus (Saunders and Rees Davies 2005; Eatwell 2006; Varga 2014; Harcourt-Brown 2014;) which predisposes to flystrike (Harcourt-Brown 2002; Meredith 2014). Low fibre diets are frequently implicated in both GI stasis (Rees Davies and Rees Davies 2003; Saunders & Rees Davies 2005; Varga 2014) and reduced caecotroph consumption (Fekete and Bokori 1985).

A low fibre level alone may not cause GI stasis but is believed to act as a predisposing factor; other factors such as stress, pain, infectious disease (e.g. coccidiosis) or medication may be required to cause clinical disease (Harcourt-Brown 2002). Provision of high fibre diets to commercially farmed rabbits reduces the incidence of diarrhoea and associated mortality rates (de Blas et al. 1986; Bennegadi et al. 2001; Blas et al. 2010) and may therefore be providing a protective function and promoting health. Reduced faecal output and faecal pellet size are often seen in the early stages of gastrointestinal stasis before faecal output ceases completely (Oglesbee and Jenkins 2012). Wild rabbits (Oryctolagus cuniculus) generally produce between 300 and 500 pellets/24 hours (Lockley 1962; Simonetti 1989; Gonzalez-Redondo 2009) although up to 820 have been reported (Taylor and Williams 1956), and mean faecal pellet diameter of wild rabbits was 7.90±0.14 mm in one study (Simonetti 1989). Diet may be responsible for these differences in numbers as, although not studied in European rabbits (Oryctolagus cuniculus), higher fibre diets significantly increase the number of faecal pellets produced by wild cottontails (Sylvilagus floridanus) (Cochran and Stains 1961). Feeding muesli and/or a lack of hay may contribute to gastrointestinal stasis and lack of caecotrophy in pet rabbits. This study aims to assess the effect of feeding two concentrate types, extruded nugget (EH) and muesli (MH) alongside ad lib hay with ad lib hay (HO) fed alone and ad lib muesli (MO) on faecal output, faecal pellet size and the quantity of caecotrophs left uneaten.

Materials and methods

This study was conducted as a part of a long term study to assess the effect of diet on the health and welfare of pet rabbits as previously described by Prebble & Meredith (2014), Prebble et al. (2015a.b) and Meredith et al (2015). Study design and methodology was approved by the Ethical Review Committees of the Royal (Dick) School of Veterinary Studies and the Food and Environment Research Agency (FERA). The rabbits were housed in a facility licensed by the Home Office; however, a project licence under the Animals (Scientific Procedures) Act 1986 (ASPA) was not required for this study. The study was continually monitored by the FERA Ethics Committee and Home Office inspector throughout its duration.

Diets

On arrival, 32 eight week old rabbits were housed in pairs and acclimatised over 40 days (days –54 to –14) by maintaining their weaning diet of 50 g per rabbit of an extruded diet (Burgess® Excel-Junior and Dwarf Rabbit; Burgess Pet Care, Thornton Le Dale, North Yorkshire, UK) once a day plus ad lib Timothy Hay provided in wall-mounted hayracks. Water was provided ad lib in 700 ml bottles. At Day –14, the rabbits were randomly allocated to one of four diet groups each consisting of eight rabbits:

1. Hay only (HO) – ad lib supply of Timothy hay (n=8);

2. Extruded diet and hay (EH) – 50 g per rabbit Burgess Excel Adult Rabbit (Burgess Pet Care, Thornton Le Dale, North Yorkshire, UK) with ad lib supply of hay (n=8);

3. Muesli and hay (MH) – 60 g per rabbit Russell Rabbit Complete Muesli (Supreme Petfoods Limited, Ipswich, Suffolk, UK) with ad lib hay (n=8);

4. Muesli only (MO) – ad lib supply (125 g per rabbit) of Russell Rabbit Complete Muesli (Supreme Petfoods Limited, Ipswich, Suffolk, UK) (n=8).

The rabbits were gradually transitioned on to the new diet over a 2-week period (Day –14 to 0) and remained on that diet for 17 months. All concentrates were weighed out daily to ensure accurate and consistent weights of food were offered. Food remaining after 24 hours was weighed to determine food consumed. The nutritional composition of the four diets offered is detailed in Table 1. Hay intake and selective feeding of muesli were recorded in months 3, 6 and 12 as reported by Prebble & Meredith (2014).

A rabbit in the EH group died suddenly on day 209, but the cause of death was not evident on post-mortem examination. A rabbit in the MO group was removed at month 7 following the development of clinical dental disease. Data from these two rabbits were not included in any subsequent analyses.

<u>Data collection</u>

Faecal samples were collected from individual rabbits in weeks 3, 9, 21 and 43. The rabbits were separated for up to three hours in the light period using a Perspex divider within their home pen. Fifteen (or the total number produced if less than 15) of these faecal pellets were then randomly selected and weighed using digital scales (Fisherbrand DP 300) and a mean faecal pellet weight calculated. The size of the faecal pellet was measured using digital callipers; as the faecal pellets are not spherical or symmetrical the longest diameter was measured.

Following the observation that longer periods of separation (>3 hrs) were required in the MO group in order to get sufficient quantities of faecal pellets, total faecal output in all groups was measured in weeks 10, 22 and 45. Bedding was removed from the pens for a 24 hour period and all faeces passed during this period collected and weighed. A sample of 20 pellets was weighed and the mean faecal pellet weight calculated. The total weight of faecal pellets produced was then divided by the mean faecal pellet weight to give an indication of the number of pellets produced.

A count of caecotrophs found in the pens during weekly cleaning out was done by the same researcher on 25 of the weeks during the period between week 4 and week 31. Caecotrophs were not measured in weeks 7 and 12 as the researcher was unable to be present during cleaning out.

Statistical Analysis

Data were analysed using Minitab (v16.1.1 © 2010 Minitab Inc.) and R software (v2.15.1 © 2012 The R Foundation for Statistical Computing). For most of the measurements, data from each time point were analysed using standard analysis of variance (ANOVA) also taking the sex of the rabbit into account, using standard Tukey's post-hoc tests to assess pair-wise differences between the groups where overall differences were obtained. Residuals were examined for adequate normality before analysis. A Pearson product moment correlation was carried out to assess the relationship between rabbit weight, faecal pellet weight and faecal pellet diameter and also between faecal weight and number of faecal pellets. $P<0{\cdot}05$ was taken to indicate the statistical significance throughout. The number of caecotrophs produced from particular time points were analysed with General linear models with Poisson errors were used to incorporate the integer nature of the caecotroph data with pairwise post-hoc Tukey analysis carried out if there were overall differences.

Results

Faecal pellet weight and diameter

Faecal pellets were consistently lighter and smaller in rabbits fed muesli only, and increased hay intake was associated with increased faecal pellet weight and size. There was a statistically significant positive linear relationship between hay intake and both faecal pellet weight ($t_{1,30}$=4.96, P<0.001, R^2=45.0) and diameter ($t_{1,30}$=4.25, P<0.001, R^2=37.6, figure 1). Faecal pellet weight was positively correlated with faecal pellet diameter at all timepoints (r>0.635, P<0.001). Rabbits fed muesli had the greatest reduction in faecal pellet size over the course of the study. Significant changes in mean faecal pellet weight and diameter occurred over the duration of the study (F3,25=3.24, P=0.039, Table 2), but this was most noticeable in the MH group (62% reduction in faecal pellet weight and 32% reduction in diameter) and MO groups (47% reduction in faecal pellet weight and 14% reduction in diameter). In contrast, smaller changes were observed in the HO (38% reduction in faecal pellet weight and 4% increase in diameter) and EH (26% reduction in faecal pellet weight and 3% reduction in diameter) (Figures 1 and 2) groups.

Significant differences were present in both faecal pellet weight and diameter at each time point (F3,25=29.92, P<0.017, Figure 2). In week three faecal pellets produced by the HO group were 26% heavier than those in the EH group and 23% heavier than the MH group, while the MO group produced pellets that were 21% smaller than the MH group (P<0.028). The EH and MH groups were not different from each other (P>0.127).

In week 9 faecal pellets produced by the HO group (0.34g±0.09, 10.53mm±1.53), continued to be significantly heavier and larger than all other groups (P<0.009) while those produced by the MO group (0.14±0.05, 8.52mm±0.85) were both smaller (P<0.002) and lighter (P<0.035). There remained no difference between the EH (0.2g±0.05, 9.72mm±0.87) and MH (0.23±0.03, 9.82mm±0.71) groups (P=0.920).

The faecal pellets in the MH group reduced in size more than those produced by other groups and by week 21 they were 29% smaller than those produced by the EH group ($P=0.011$) and by week 43 pellets produced by the MH group were 47% smaller than the EH group and were similar in size to those produced by MO group ($P=0.90$, Figure 3).

Total faecal output

Increased hay intake was associated with increased number of faecal pellets, with a positive linear relationship between hay intake and both the weight of faeces produced ($t_{1,13}=3.45$, $P<0.004$, $R^2=47.7$) and the number of faecal pellets ($t_{1,13}=3.11$, $P<0.008$, R2=42.6).Significant differences in the number of pellets produced over a 24 hour period were present between groups at all time points ($F_{3,11}=18.89$, $P<0.003$, Table 3).
The HO group produced 46 - 62% more faecal pellets over a 24-hour period than those in the MO group ($P<0.001$), 24-29% more than the EH group ($P<0.07$) and 32-43% more than the MH group ($P<0.02$). The EH produced (12- 25%) more faecal pellets than the MH group although this difference was not significant ($P>0.06$). Likewise there was no difference in the number of faecal pellets produced by the MH and MO groups ($P>0.13$, Figure 4).

In week 52 a single rabbit in the MO group had signs of gastrointestinal stasis including reduced food intake, hunched posture, tooth grinding, reduced faecal pellet production and altered behaviour and was removed from the study for treatment.

Caecotrophs

Uneaten caecotrophs were found in greatest frequency in rabbits fed muesli. No caecotrophs were found in pens in the HO group for 11 of the 25 weeks where counts were performed, and the caecotrophs found in the HO group in the remaining 14 weeks were only from one pen. Analysis was therefore only performed on the three remaining groups. Overall, significant differences between groups were seen throughout the trial ($\chi^2_{3,15}=5.35$, $P<0.001$, Figure 5). The MO group left more caecotrophs (18±2) than the EH (4±3) and MH

(7±1) in week four ($P<0.001$), whilst no difference was present between the EH and MH groups ($P=0.273$). However, by the end of the trial the reduction in the number of caecotrophs left by the EH group (1±1) was significantly less than those left by the MH group (5±2, $P<0.02$). While the number of caecotrophs left by the MO group had fallen (14±3) this was still significantly more than all other groups ($P<0.001$).

Discussion

This study demonstrates that diet has a statistically significant impact on faecal pellet weight and size, total faecal output and caecotrophy. Significantly heavier and larger faecal pellets were produced by those rabbits consuming hay, in contrast to the significantly smaller, lighter pellets produced by the MO group, which had no access to hay. Faecal pellets produced by the MO group were similar in appearance to the small irregular pellets observed in the early stages of GI stasis (Lord 2012, Harcourt-Brown 2014). Although the mean faecal diameter in the MO group was comparable with that reported in wild rabbits of 7.90mm, the range included pellets as small as 5.80mm (Figure 3). As low fibre levels are frequently implicated in GI stasis in pet rabbits (Varga 2014; Rees Davies and Rees Davies 2003; Lord 2012) the finding that low hay diets are associated with reduced faecal pellet diameter and faecal output is of clinical relevance.

The smaller faecal pellets produced by the MO group throughout the study and the MH group towards the end of the study are similar in size appearance to those observed by the authors and described by Harcourt-Brown (2014) in early clinical cases of gastrointestinal stasis. However, the low occurrence of GI stasis (one rabbit) in this study supports the assertion that low fibre diets are only one contributing factor in the development of GI stasis (Harcourt- Brown 2002) and that low fibre diets are not directly causative. Dietary fibre has a protective effect on the morbidity and mortality from digestive diseases observed in farmed rabbits and at weaning (Bennegadi et al. 2001; Gidenne et al. 2001) this may also be true for

GI stasis. This protective effect may be a result of the effect of dietary fibre on volatile fatty acid (VFA) production. Rabbits fed high fibre, low starch diets produce less VFA's than those on low fibre, high starch diets (Carabaño et al, 1988; Gidenne et al, 2004). This would suggest that rabbits fed the low fibre, high starch muesli diet would have higher VFA levels which have been associated with reduced colon motility in in vitro studies (Squires et al. 1992; Dass et al. 2007). This reduction in motility may make rabbits fed on a muesli only diet more susceptible to the development of GI stasis if other factors associated with reduced motility such as pain or stress (Harcourt-Brown, 2014) are present.

Faecal output over a 24 hour period was greater in rabbits consuming more hay (hay intake reported in Prebble and Meredith (2014)). As large fibre particles pass rapidly through the colon and are excreted in faecal pellets (Jilge 1982; Carabaño et al. 1988), the differences in hay (and therefore fibre) intake between the groups can explain these difference in faecal output. The number of faecal pellets produced by the EH and MH groups fall within the lower end of the range (300-500 daily) generally reported in wild rabbits (Lockley 1962; Simonetti 1989; Gonzalez-Redondo 2009). The HO groups produced numbers closer to, or exceeding, the top end of this range, but did not reach levels in wild rabbits reported by Taylor and Williams (1956). The MO group had considerably lower faecal output, similar to the findings of Arnold and Reynolds (1943) in Arizona and Antelope jack rabbits fed a highly digestible mash, which produced half as much as those fed grass. In addition, differences in faecal pellet colour were observed between groups; the HO group produced faecal pellets that were lighter in colour, whilst those produced in the MO group were darker. The faecal pellets produced by the EH and HO groups were similar in colour. Objective quantification of these differences, for example using colorimetry, was outside the scope of this project.

Uneaten caecotrophs were regularly found in all groups fed concentrates but infrequently in the HO group. Highest numbers were found in the MO group (up to 30), whilst the MH (up to 10) and EH (up to 5) groups that consumed hay left less caecotrophs, supporting findings that caecotroph consumption is associated with forage based (Clauss et al. 2012) and high fibre, low protein diets (Fekete and Bokori 1985). Caecotrophy contributes to protein, energy and vitamin requirements (Kulwich et al. 1953; Fraga et al. 1991; Kerti et al. 2005), and the higher protein and energy content of concentrates allows nutritional requirements to be met solely from the diet, resulting in increased numbers of uneaten caecotrophs. Other factors implicated in reduced caecotroph consumption include obesity, perineal dermatitis, dental and musculoskeletal diseases (Harcourt-Brown 2014). Obesity was observed in the MO group (Prebble et al. 2015), but was not sufficient to prevent grooming (observed frequently by the researcher) and should not have impaired caecotrophy. No other conditions were present in the rabbits before or during data collection. In the absence of other contributory factors, the differences in uneaten caecotrophs within the pens could be attributed to dietary factors alone. The cause of the reduction in uneaten caecotrophs in the MO group by the end of the trial is not known, but possibilities include an age-related effect, or changes in dry matter (DM) or water intake and would require further investigation..

Smaller faecal pellet size and weight, lower total faecal output and greater number of caecotrophs not consumed may indicate that muesli based diets contribute to the development of digestive conditions. In particular, feeding muesli in the absence of hay cannot be recommended. Further study is required to assess the significance of diet in the development of gastrointestinal stasis and the interaction of other factors. A higher fibre diet promotes a larger faecal pellet size and weight and higher total faecal output, therefore feeding hay alone or alongside concentrate diets is recommended for pet rabbits and may be beneficial in preventing protective against GI stasis.

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Figure legends

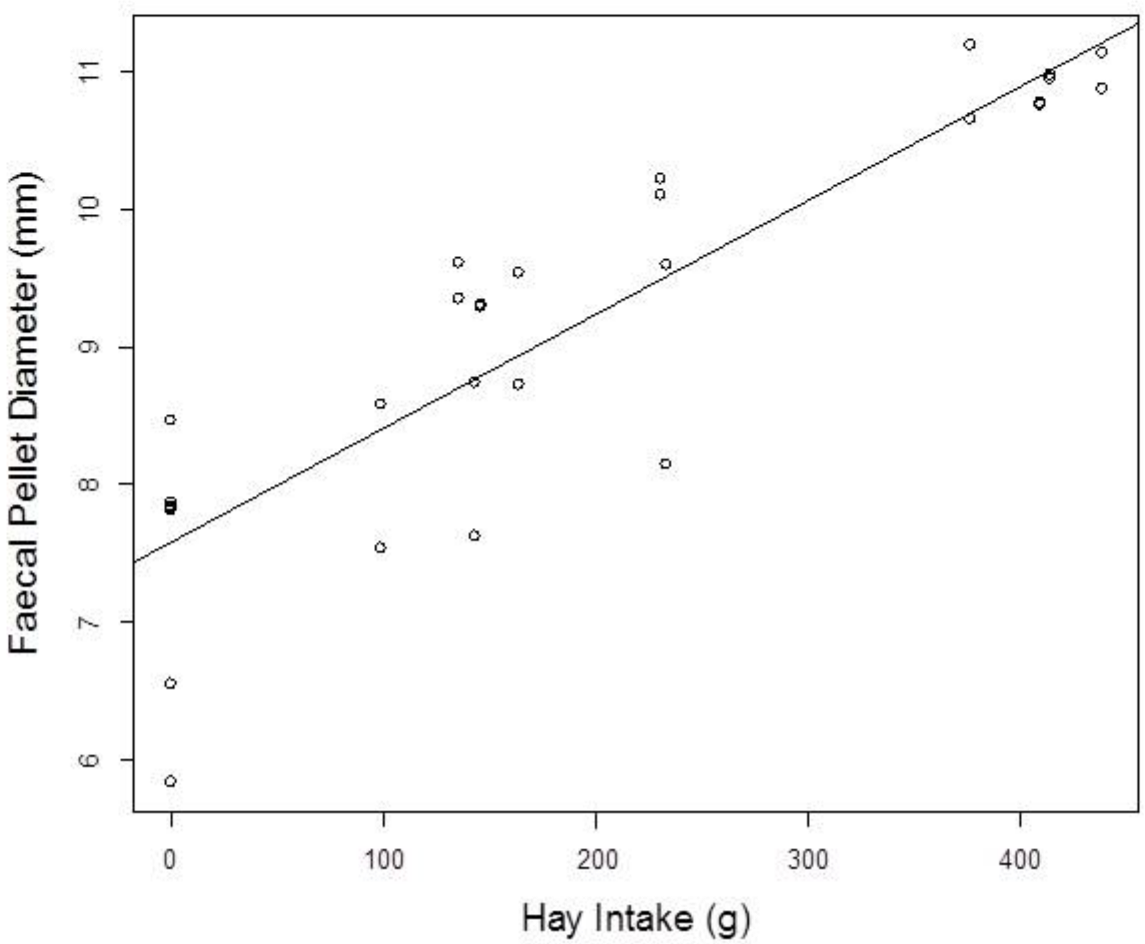


Figure 1 Scatter plot of hay intake (g) by pen and faecal pellet diameter (mm) of Dutch rabbits (n=30) fed four diets (HO, EH, MH and MO) in week 43 of the trial period with linear regression line fitted to data.

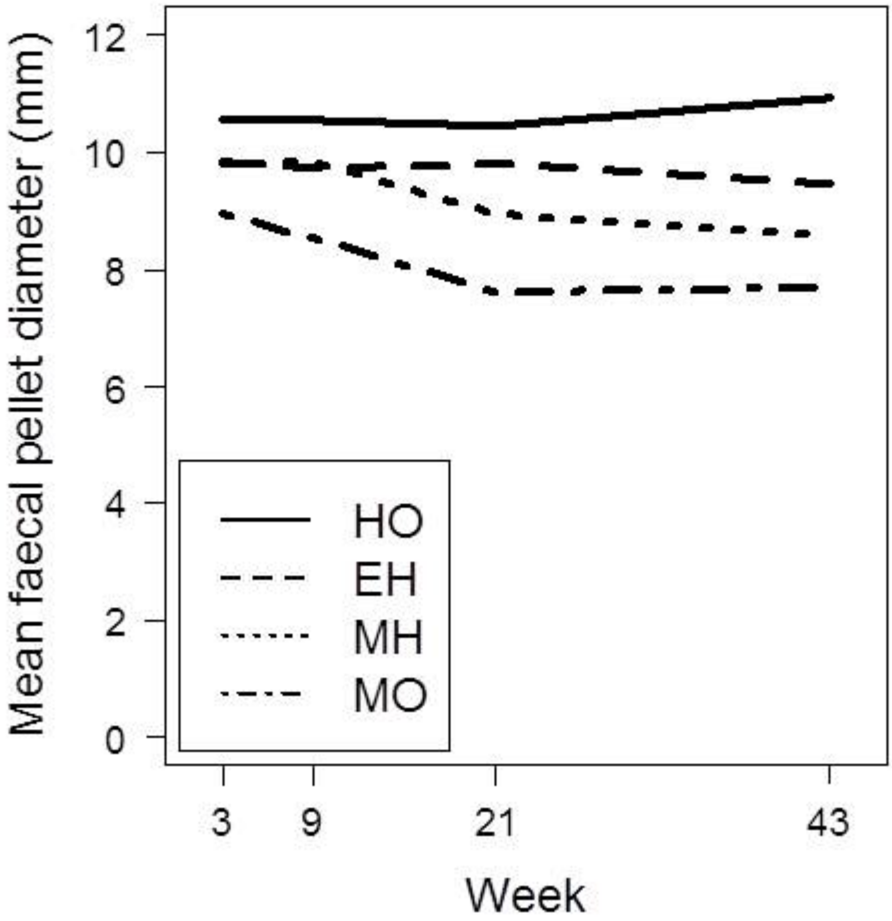
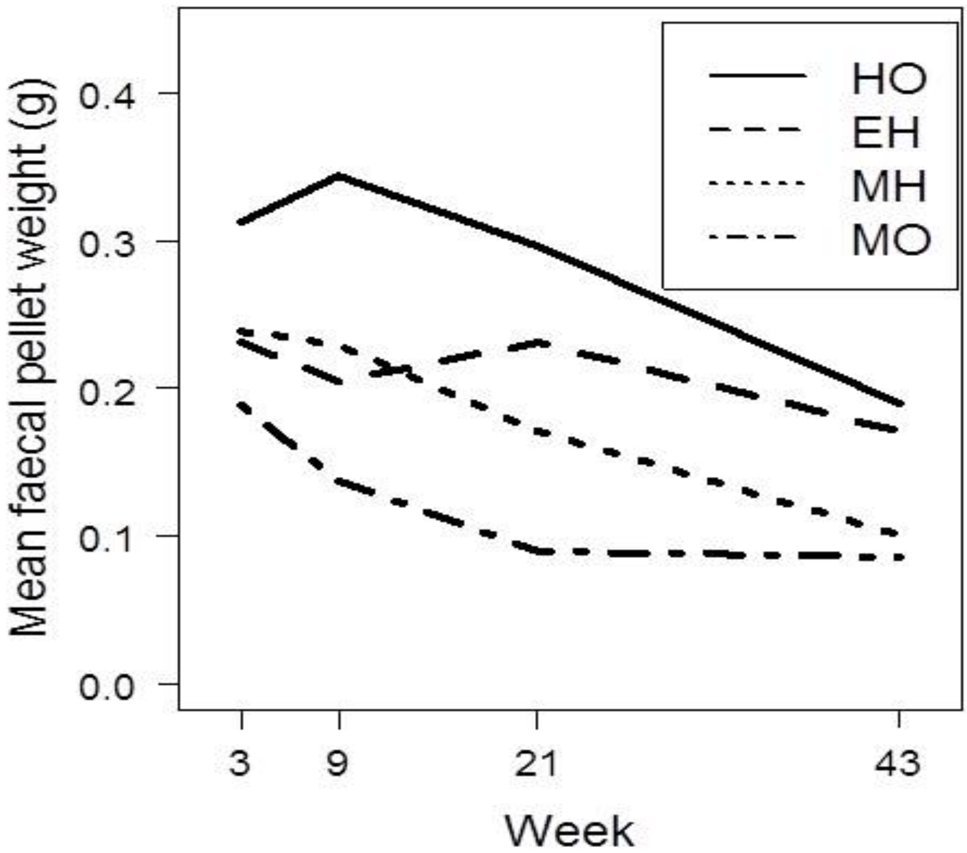


Figure 2 a) Mean faecal pellet weight (g) of Dutch rabbits (n=32) fed four diets (HO, EH, MH and MO) measured at four points over a 40 week period.



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465 b) Mean faecal pellet diameter (mm) of Dutch rabbits (n=32) fed four diets (HO, EH, MH and

466 MO) measured at four points over a 40 week period.

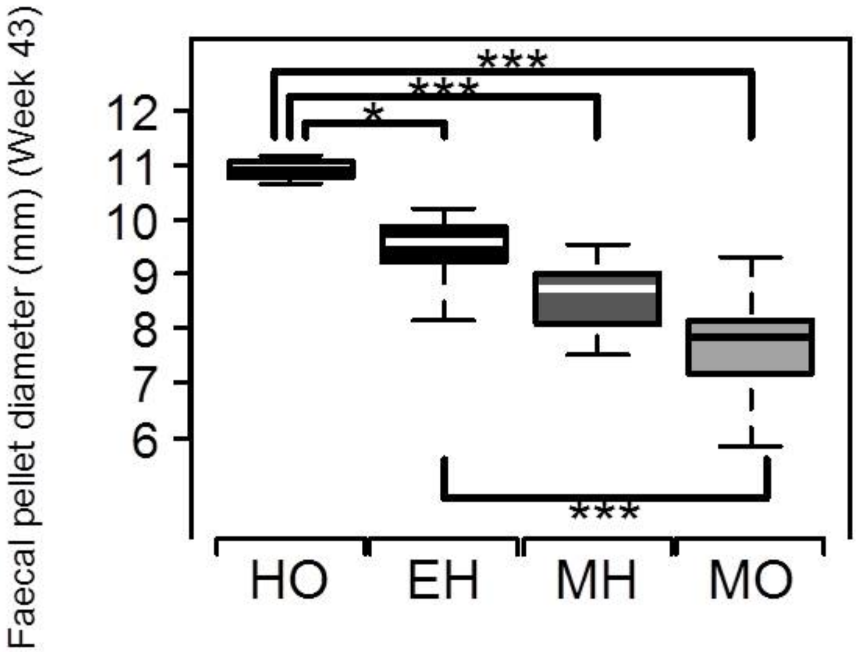

Figure 3 Boxplot of faecal pellet diameter (mm) of Dutch rabbits (n=30) fed four diets (HO, EH, MH and MO) in week 43 of the trial period. The horizontal bar represents the median, the box is the interquartile range and whiskers represent the range of values. *** significant at P<0.001, * significant at P<0.05

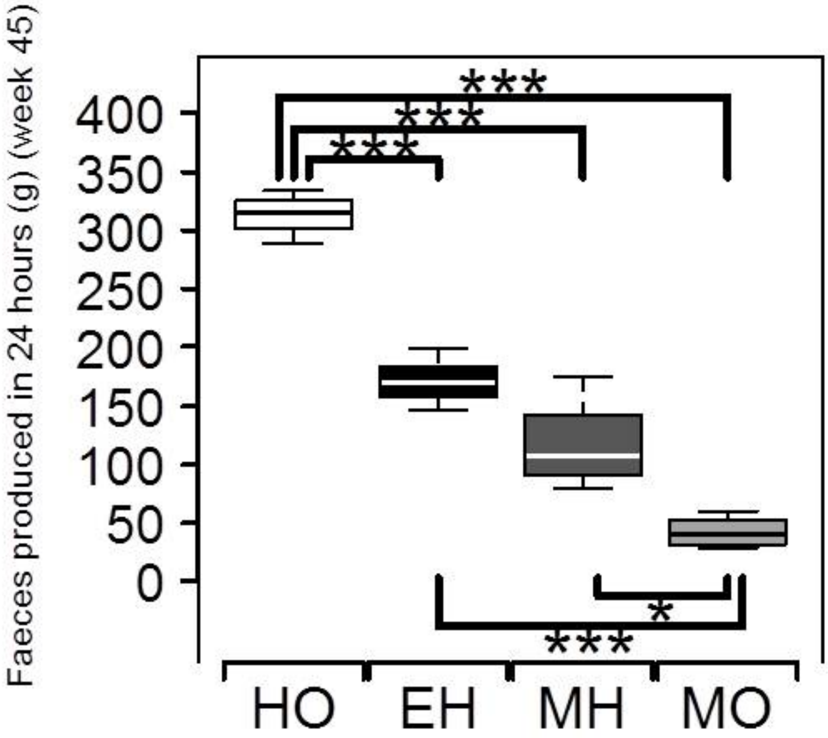

Figure 4 Boxplot of weight of faeces produced over a 24 hour period (g) by Dutch rabbits (n=30) fed four diets (HO, EH, MH and MO) in week 45 of the trial period. Boxplots as for Figure 3. *** significant at P<0.001, * significant at P<0.05

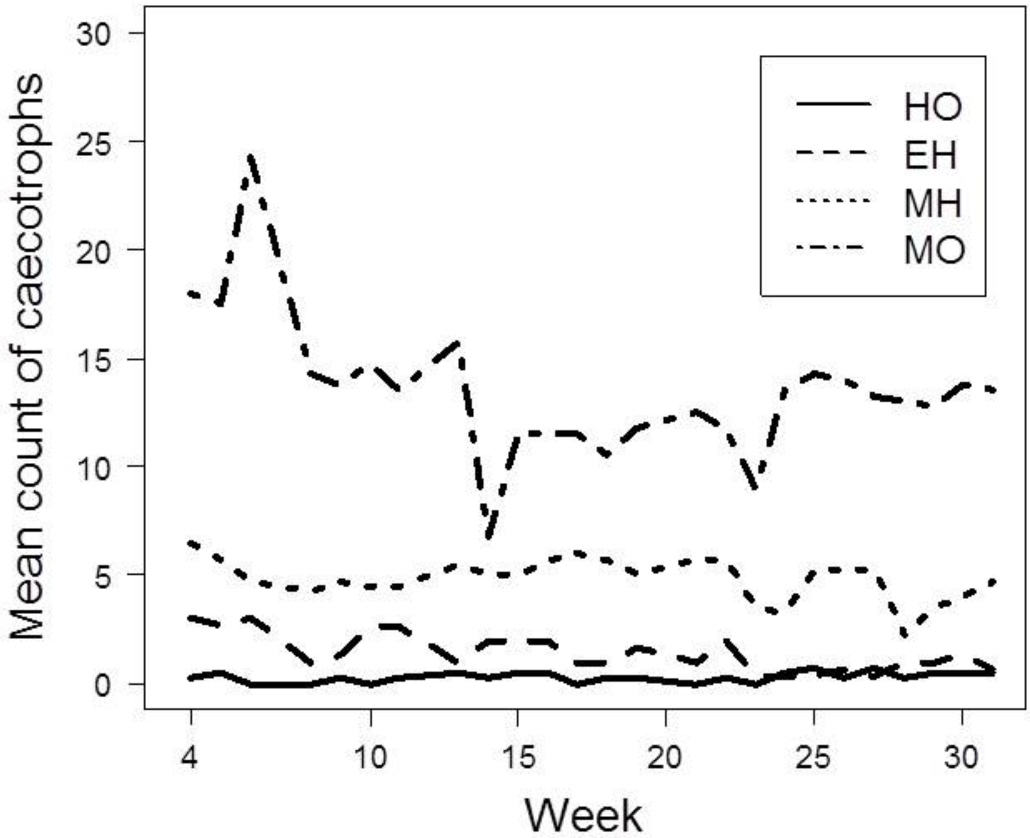

Figure 5 Mean number of caecotrophs found on a weekly basis in the pens of Dutch rabbits (n=32) fed four diets (HO, EH, MH and MO) measured at over a 40 week period.

Table 1-Nutrional composition of diets offered with values expressed as %DM

	Timothy Hay	Extruded Nugget[a]	Muesli[b]
Crude Protein (%)	9	13	14
Fat(%)	2	3	2.5
Crude Fibre(%)	29	19	14
Ash (%)	6.5	5.5	5
NDF[c] (%)	60	38	29
ADF[d] (%)	33	21	20
Calcium (%)	0.3	0.6	0.6
Phosphorus (%)	0.22	0.51	0.4
Ca:P Ratio	1.36	1.18	1.5

[a]Burgess Excel -Adult Rabbit(Burgess Pet Care, Goole, East Yorkshire, UK)
[b] Russell Rabbit-Complete Muesli(Supreme Petfoods Limited, Ipswich, Suffolk, UK)
[c] Neutral Detergent Fibre
[d]Acid Detergent Fibre

Table 2- Mean size and weight of faecal pellets produced in each group at each time point

	Week 3	Week 9	Week 21	Week 43
HO	0.31g±0.07	0.34g±0.09	0.3g±0.04	0.19g±0.03
	10.53mm±0.71	10.53mm±1.53	10.43mm±0.78	10.91mm±0.18
EH	0.23g±0.03	0.2g±0.05	0.24g±0.05	0.17g±0.03
	9.82mm±0.58	9.72mm±0.87	9.83mm±0.8	9.44mm±0.69
MH	0.24g±0.06	0.23g±0.03	0.17g±0.03	0.09g±0.04
	9.80mm±0.7	9.82mm±0.71	8.93mm±0.8	8.67mm±0.75
MO	0.19g±0.04	0.14g±0.05	0.09g±0.02	0.1g±0.03
	8.93mm±0.78	8.52mm±0.85	7.61mm±0.96	7.67mm±0.91

Table 3- Mean number of faecal pellets produced by a pair of rabbits in each group at each time point

	Week 10	Week 22	Week 45
HO	983 ±128	1040±37	1058±128
EH	751±99	784±49	753±70
MH	660±128	586±145	618±186
MO	534±114	472±76	402±65